THE

AMERICAN REVIEW OF TUBERCULOSIS
ABSTRACTS OF TUBERCULOSIS

VOLUME XXVII MARCH, 1933 ABST. NO. 2

CONTENTS

PAGES

Pathology

a. *Anatomical and microscopic (concluded)* 25–28
b. *Physiological* 28–30
c. *Bacteriological: tubercle bacillus* 30–37
d. *Experimental: immunity: allergy: tuberculin* 37–48

Blood-Vessels in Tuberculous Cavities.—This is an extensive and detailed study on the pathological changes in the blood-vessels in cavity-walls. The literature is extensively reviewed. The alterations seen in blood-vessels may be grouped into those of defence and those of destruction. The ridges which are frequently seen in cavities do not always contain blood-vessels. The innermost layer of cavities is generally composed of necrotic tissue. In this layer the changes are essentially those of destruction and necrosis. But, before the blood-vessel is destroyed, it is usually completely obliterated. The essential changes are connective-tissue proliferation in the adventitia, thickening and increase in the elastic fibres of the media, and proliferation of endothelium and elastic fibres leading to the obliteration of the lumen in the intimal layer. These processes occur chiefly in the outer layers of the cavity-wall, while in the inner layer the proliferated tissue elements undergo necrobiosis. In no case was any specific tissue change found in the blood-vessel wall. Even in the rare cases in which tubercle bacilli could be demonstrated in the media or lumina of blood-vessels, specific tubercles were absent. One of the most important points of the study is the regularity with which complete vascular occlusion was observed wherever blood-vessels were near to destructive processes. This occlusion is regularly achieved by proliferation of the intima, and not by thrombosis. The vessels may contain small accumulations of blood cells, usually with a large proportion of white cells; tubercle bacilli are often found in these cellular aggregates, but thrombosis is absent. In spite of the absence of tubercle bacilli intimal tubercles were never seen. These findings make it likely that the tubercle bacilli gain entrance into the blood-stream at distant points and that they do not invade the vascular wall, since they have never been found in the intima.—*Über die Veränderungen an den Blutgefässen im Bereich tuberkulöser Lungenkavernen, M. Kasper, Beitr. z. Klin. d. Tuberk., September, 1932, lxxx, 537.*—(*M. P.*)

Bands and Ridges in Pulmonary Artery.—Cord-like structures in the vascular system are rare, and even more infrequent is their presence in the branches of the pulmonary artery. Only two references to this condition were found in the literature. Two cases are reported in detail. A man of 60, with diffuse arteriosclerosis, showed band-like structures in the pulmonary arteries. These consisted of young and old connective tissue, with hyalinization, endothelial cells, lymphocytes, and red blood cells and pigmented areas. Newly formed blood-vessels and blood sinuses were found throughout. The bands themselves were lined by intimal lining cells. In short, the bands represented organized and organizing thrombi. The second case, a man of 49, also showed diffuse arteriosclerosis. Ridges were found in the pulmonary artery, and

histological examination showed them to be organized mural thrombi covered by intimal lining cells. The findings in these cases are interpreted as being due to thrombi forming on areas of the intima roughened by arteriosclerotic changes. There was no evidence to support a diagnosis of Ayerza's disease, and it was felt that congenital anomalies, as the underlying cause, could be ruled out.—*Bands and Ridges in the Pulmonary Artery, O. Saphir, Arch. Path., July, 1932, xiv, 10.*—(*R. D.*)

Early Cellular Reactions to Tubercle Bacilli.—The character of the cellular response to infection with tubercle bacilli has been the subject of long-continued, active investigation. Special emphasis in recent years has been laid on the monocyte which is believed to be the precursor of the epithelioid cell. This emphasis has tended to draw attention away from the cell long known to be the first responding element to infection with tubercle bacilli, but not considered of much significance in tubercle-formation, namely, the neutrophilic polymorphonuclear leucocyte. That the early response of this cell is of genuine importance in the formation of the tubercle has recently been shown by Vorwald. He found that after intravenous injection in rabbits of human tubercle bacilli the intracapillary leucocytic response in the lungs was intense in one hour, and even at this early period it was difficult to find a tubercle bacillus not taken up by a polymorphonuclear leucocyte. His results indicate that cells of this group concentrate the bacilli into small sharply localized cellular masses, and that these masses are the groundwork on which the tubercle develops. As the hours go by, large mononuclear exudate cells gradually infiltrate the polymorphonuclear mass, phagocytose the cells of this mass, and at the same time take over into their own cytoplasm the tubercle bacilli previously ingested by the neutrophilic leucocytes. An almost complete replacement thus takes place, so that at twenty-four hours a tubercle is seen that is predominantly mononuclear, with the tubercle bacilli almost exclusively in cells of this class. Subsequent modifications of these cells, by nuclear and cytoplasmic changes, convert them into typical epithelioid cells. The results just described occur in primary infection. It is well known that previous infection with tubercle bacilli or previous treatment with dead tubercle bacilli modifies the course of subsequent infection with virulent bacilli. Animals thus treated not only react positively to tuberculin, but possess a considerable degree of immunity. Moreover, in animals treated with dead bacteria, in addition to the general immunity, an exceptionally high local immunity has been demonstrated. In view of all these facts it seemed desirable to supplement Vorwald's experiments on primary infection with a study of the reaction at early hours in animals whose reacting capacity had been modified by previous infection and artificial immunization. The testis of the guinea pig was chosen for this experiment because (1) it probably exhibits the allergic reaction of reinfection with a higher degree of intensity than any other organ of the body, (2) the location and isolated character render it especially suitable for an experiment demanding exact and equal dosage, and (3) the choice of this organ makes possible a comparison of the effects of general and local immunization on the development and course of a subsequently induced local tuberculosis. For this phase of the experiment, one testis was locally immunized by several injections of dead tubercle bacilli. Animals of this group thus could be used for a comparative study of the effects of local and general immunization in the same animal, the reaction to live tubercle bacilli in the immunized testis serving as an index of the effect of local immunization, and the reaction to an equal dosage of bacilli in the other testis, as an index of the degree and character of general immunization. *Experiments:* Three groups of guinea pigs were used: (1) normal guinea pigs, (2) guinea pigs rendered tuberculous by an injection of virulent human type tubercle bacilli (H37) one month previously into the left axilla, and (3) guinea pigs that had been treated by 5 injections of 0.1 mgm. of a suspension of heat-killed human tubercle bacilli (H37) at intervals of two and four days, and that had been allowed to rest one month after the fifth injection. These injections of dead bacilli caused extensive scarring with the production of a moderately vascular granulation tissue extensively infiltrated by cells of inflammation, especially lymphocytes and large mononuclear exudate cells. The animals then were treated as follows: 0.1 mgm. of H37 tubercle bacilli per kilogram of animal weight was injected into the left testis in groups 1 and 2, and into both testes in group 3. Animals of each group were then killed at the end of one, three, six, ten, sixteen, twenty-four, forty-eight and seventy-two hours. The testes into which the injection had been made were fixed, sectioned and stained *Results:* The cellular reactions in these four states of testis tissue (normal testis of the normal animal, normal-appearing testis of the tuberculous animal, scarred testis of the locally immunized animal and the normal-appearing opposite testis of the same animal) were studied. In all cases,

the most actively reacting cell at the early hours was the polymorphonuclear leucocyte. Phagocytosis of bacilli by these cells was practically complete in three hours. The most intense exudation of these cells at the early periods occurred in the testes of the tuberculous animals. In this group a great deal of fibrin appeared in the exudate, and at the same time a rapid degeneration of the seminiferous tubules occurred (changes characteristic of the tuberculin reaction in the testis and due to the protein of the tubercle bacillus). The next most intense exudation of polymorphonuclear leucocytes occurred in the guinea pigs treated with repeated injections of dead tubercle bacilli. The reaction was of approximately equal intensity in the locally immunized testis and the opposite testis modified in reactive capacity only by the general immunization conferred by the local immunity procedure. The least intense exudative reaction occurred in the testes of the normal animals. Between twenty-four and forty-eight hours after the inoculation with live bacilli, a gradual replacement of the polymorphonuclear by large mononuclear exudate cells occurred in all animals. Extensive phagocytosis of polymorphonuclear leucocytes by the large mononuclears took place, resulting in a transferrence of tubercle bacilli from the cytoplasm of the polymorphonuclears to that of the large mononuclears. This rôle of the large mononuclear leucocytes did not appear earlier in the locally immunized testis than in the other testes of the series. At seventy-two hours the familiar picture of early mononuclear cell tubercles was present, each of these tubercles representing a previous site of focalization of polymorphonuclear leucocytes and bacilli. Maturation of these large mononuclears to the typical epithelioid appearance had not yet taken place. *Conclusions:* Prompt localization of tubercle bacilli is brought about by exudation of polymorphonuclear leucocytes and phagocytosis of bacilli by these cells. Aggregation of the leucocytes, containing all the bacilli, into distinct and separate foci soon occurs. The site of future true tubercle-formation is thus determined, for the polymorphonuclears are soon replaced by large mononuclears which later develop into epithelioid cells. The fact that this replacement did not occur appreciably earlier in the locally immunized testes, already full of mononuclear cells, than in the other testes of the series suggests that the large mononuclear cells concerned in the reaction came directly from the blood-stream and were not derived locally. An already present tuberculosis in the animals greatly accelerates the reacting capacity of the polymorphonuclear leucocytes. This may be looked on as simply one feature of a typical tuberculin reaction. Previous inoculation with dead bacilli brings about a similar allergic state, although the acceleration and increased intensity of inflammatory reaction over that seen in the normal animal, are not nearly so great as in the truly tuberculous animal.—*Early Cellular Reaction to Tubercle Bacilli, A Comparison of this Reaction in Normal and Tuberculous Guinea Pigs and in Guinea Pigs Immunized with Dead Bacilli, E. R. Long, A. J. Vorwald and L. Donaldson, Arch. Path., December, 1931, xii, 956.—(J. S. W.)*

Stem-Cell of Monocyte.—The origin of the monocyte has been assigned variously to the myeloblast, the common or the specific endothelium, the lymphocyte and an undifferentiated mesenchymal cell. Naegeli considered the cell a member of the myeloid series arising from the myeloblast. McJunkin and others expressed the belief that the monocyte is derived from common endothelium. Aschoff and Kiyono placed the monocyte in close relation with the specific endothelium (reticulo-endothelium) and its derivatives, the histiocyte or macrophage. Maximow and Bloom considered the monocyte to arise through intravascular transformation of the small lymphocyte, but Maximow has admitted the possibility of an origin from the histiocyte or from an undifferentiated mesenchymal cell. Cunningham, Sabin and Doan described an origin of the monocyte from a primitive blood cell that itself is closely related to undifferentiated mesenchymal elements in the hematopoietic organs. Forkner believes that monocytes arise from similar cells, passing through a stage, which he calls a premonocyte, into the monocyte. A case of monocytic leukaemia gave an opportunity to study the nuclear structure of the monocyte in detail by use of a silver-impregnation method of staining blood-films. By this method the monocyte has been traced to a stem-cell corresponding to the haemohistioblast of Ferrata. A graded series of intermediate forms exists between the round or oval lace-like nucleus characteristic of the haemohistioblast and the folded, skein-like nucleus of the mature monocyte. The haemohistioblast is found almost constantly in myelogenous leukaemia and has been described in lymphatic leukaemia and in monocytic leukaemia; the cell has been shown to be the circulating stem-cell in the prehepatic period of the formation of blood in the embryo and the megaloblast has been traced to the same cell. Further, the cell corresponds in nuclear type to the

mesenchymal cell, the logical form of a multipotential element. The present study would appear to strengthen the position of the haemohistioblast as a true stem-cell. Accepting the observation of Ferrata that the haemohistioblast corresponds to the cell of the embryonic mesenchyme, this study consequently would favor the view held by Cunningham, Sabin and Doan and Forkner that the precursor of the monocyte is an undifferentiated mesenchymal cell. The observations reported do not define the limits of the tissues from which cells of mesenchymal type may arise. They do not preclude the possibility of the reticulo-endothelium, under the stimulus of proliferation, being this source.—*The Stem Cell of the Monocyte, J. F. Rinehart, Arch. Path., June, 1932, xiii, 889.—(J. S. W.)*

Tuberculosis of Lung with Apices Uninvolved.—A man, 30 years of age, died of pulmonary tuberculosis about six weeks after the clinical onset of his disease. Autopsy showed that both apices were free from tuberculous foci. Such cases prove that the apical onset of tuberculosis is not a rule without exception, and that acute foci may develop without lesions in the apices from which bacilliferous material may be aspirated into the lower parts of the lungs.—*Ueber einen weiteren Fall von kavernöser Lungenschwindsucht ohne Beteiligung der Spitzen. Ein Beitrag zur Frage des infraclaviculären Beginns der tertiären Lungentuberkulose, Berta Elias-Orth, Beitr. z. Klin. d. Tuberk., June, 1932, lxxx, 175.—(M. P.)*

Tests for Respiratory Efficiency.—The various tests which have been employed for estimating the efficiency of the respiratory apparatus are described and briefly discussed. First, there are various elementary points which can be determined on a routine clinical examination, such as respiratory rate, whether the accessory muscles of respiration are in use, size of chest, degree of pathological lesion, together with blood-counts, especially estimation of haemoglobin, and possibly also determination of blood-gases in arterial and venous blood. So many factors are concerned in producing deviations from the normal in any of these methods that results obtained are necessarily haphazard and in many instances quite unsatisfactory. The second series of tests is concerned with measurements of the vital capacity and its component parts, the tidal air, supplementary air, and complementary air. Little training is necessary for this test, but the disadvantage lies in the difficulty of knowing the normal standards for individual patients. No measurement of lung-volume can be complete without a knowledge of the residual air, the estimation of which requires a more complicated proceeding, not always applicable to the sick patient. Third, there are the tests for respiratory efficiency concerned with various "indices" devised principally to give some idea of the ventilating power of the respiratory apparatus. These indices have the grave disadvantage that normal figures provide too rigid a standard, and wide deviations may be possible in health. Lastly come determinations of the expiratory force and sustaining power of the respiratory apparatus as tested with the mercury U-tube of Flack. Of 103 patients, presumably healthy as regards their respiratory apparatus, the average expiratory force for men was 88 mm. of mercury, and for women 58 mm. The standard for the Air Force is 110 mm. and under 80 mm. is considered to indicate respiratory insufficiency. In the endurance tests 3 men failed to keep the mercury at 40 mm. for longer than a few seconds. The remaining 36 gave an average of 33 seconds, while the Air Force standards require 50 to 60 seconds. The pulse response is also important in this test and a rise from 72 to 132 or 144 is regarded as unsatisfactory. So many factors enter into the results of these tests, psychological as well as the efficiency of other systems and parts of the body such as the abdominal muscles, that, taken alone, these tests would appear to have little value for the special purpose of investigating respiratory efficiency before thoracic surgery is carried out. In any case, the standards set by Flack for the Air Force are too high for the ordinary population. Standards are suggested, but the number of patients is considered too small for a satisfactory basis.—*Tests for Respiratory Efficiency with Special Reference to the Mercury U-Tube, A. Moncrieff, Lancet, September 24, 1932, ccxxiii, 665.—(E. H. K.)*

Respiration and Gaseous Metabolism. Circulatory system, respiration and blood represent the functional unit to supply the cells with oxygen and to eliminate the carbon dioxide. A failure of this total system of transportation is rare except in preagonal conditions. Impairment of one part of this functional entity is frequent, but it usually does not result in a reduction of the gaseous metabolism because of the multiplicity of available compensatory mechanisms. In order to gain a complete picture of the function it is necessary to determine simultaneously the following factors: oxygen consumption and carbon-dioxide excretion,

oxygen and carbon-dioxide capacity of the blood and the actual amount of these gases in the blood; respiration at rest and maximally effective respiration; the spirographically determinable oxygen deficit. The technical and methodical problems of such determinations are discussed in detail. A large number of patients and normal persons was examined, and it was found convenient to differentiate five groups of respiratory insufficiency, as follows: (1) centrally caused insufficiency; (2) caused by impairment of respiration itself; (3) impairment of air and blood distribution within the lung; (4) exclusion from ventilation of part of the lung (short circuit); (5) so called pneumonosis of Brauer. A number of clinical examples are presented in each of the above groups. Both local cerebral lesions and certain forms of poisoning belong to group one. The second group is the most important one from a clinical point of view; it included bronchial stenoses, asthmatic attacks, infiltrations of pulmonary parenchyma, fibroses, pleural effusions, pulmonary oedema, pneumothorax, immobilized thorax, etc. If, in such cases, the respiratory reserves are sufficient, the blood gases are normal; if the reserves are exhausted to maintain normal function at rest, dyspnoea exists and the oxygen saturation of the arterial blood is incomplete. Cases characteristic of group (3) can be caused by an impairment of the balance between respiratory volume and residual air, such as in emphysema with the marked increase in residual air and decrease in vital capacity; it can be caused by an imbalance of respiratory volume to the dead space (for example, in mediastinal flutter); or by an uneven distribution of respiratory air in the lung and a disturbance in the distribution of the blood in the pulmonary circulation. A short circuit is produced when the circulation continues through a collapsed portion of the lung so that normal oxygenation is prevented. Pneumonosis is defined as a decrease in the permeability for oxygen of the respiratory parenchyma. Brauer found such a concept necessary to explain the early cyanosis in influenza patients in whom no pulmonary or circulatory involvement existed. The following therapeutic deductions are made: Oxygen therapy is useless in patients without oxygen deficit. Patients belonging to group (1) and (5) should be treated with oxygen. Group (2) needs oxygen when the respiratory reserves are low. Respiratory arhythmias, except cerebrally caused ones, should always receive oxygen therapy.—*Klinische Untersuchungen über Atmung und Blutgase, K. Jansen, H. W. Knipping and K. Stromberger, Beitr. z. Klin. d. Tuberk., August, 1932, lxxx, 304.*—(*M. P.*)

Pulmonary Gas Absorption.—Gases and anaesthetic vapors contained in alveolar cavities shut off by complete bronchial obstruction gradually leave the lung and finally disappear so that the lung becomes atelectatic. The speed of the disappearance of these gases is proportionate to their solubility coefficient, diffusion speed, and chemical affinities for substances dissolved in the blood. Since ligature of the branches of the pulmonary artery corresponding to the obstructed lung prevent this disappearance of gases and vapors from the alveoli, it has been concluded that this disappearance is due to absorption by the blood circulating through the lung. The results obtained in closed as well as open chest experiments show that within 2 to 7 minutes after bronchial obstruction the oxygen percentage falls rapidly from 15 to 5 or 6 per cent. It remains at these figures until complete disappearance of the alveolar air. In animals in which gas analyses were performed until complete atelectasis occurred, the curves plotted for percentages vary inversely. It is thus seen that the entrapped alveolar air rapidly undergoes marked quantitative changes, the percentage of oxygen dropping and carbon dioxide rising, so that their respective partial pressures tend to come into equilibrium with the corresponding gases of the venous blood. These changes occur in exactly the same way in animals as in man. Determinations which were carried out by the same technique for anaesthetic vapors and gases, namely, ether, ethyl chloride, nitrous oxide and ethylene, showed the great rapidity of their absorption. Integrity of the alveolar endothelium is just as necessary as integrity of the pulmonary circulation. Oedema of the lung, produced by injection of concentrated ether vapor into the lung, instantaneously stops gas absorption. Comparative study of absorption by the pleural cavity of oxygen, carbon dioxide, nitrogen, air, hydrogen, and helium showed that their absorption is regulated by the same physicochemical laws governing absorption of gases from the obstructed lung. The aim of this investigation has been to offer direct experimental proof that complete bronchial obstruction is the exclusive cause of atelectasis.—*Studie in Pulmonary Gas Absorption of Oxygen, Carbon Dioxid, Nitrogen, Hydrogen, Helium, Ethylene, Nitrous Oxid, Ethyl Chlorid and Ether in the Lung. With Some Observations on Pleural Absorption of Gases, P. N. Coryllos and G. L. Birnbaum, Am. J. M. Sc., March, 1932, clxxxiii, 317.*—(*W. I. W.*)

Tuberculosis and Feeding.—The acid, base and ion relations in metabolism in tuberculosis were studied in animal experiments. Acid-fed guinea pigs gained least in weight and were more susceptible to the usual secondary infections. The alkali-nourished animals showed a definite increase of alkali reserve of the blood, while the acid-fed animals showed a decrease. After 9 months' treatment the animals were infected with tubercle bacilli and the alkali-fed animals survived longest while the acid-fed lived, on the average, 30 days less than the normally nourished animals. The withdrawal of sodium chloride had no influence upon the tuberculosis. No anatomical differences were found between the animals of the various groups, but it appears that the acid foods diminish the resistance of the animals, without, however, materially affecting the course of the tuberculosis.—*Tuberkulose und Ernährung. Physiologische Tatsachen und experimentelle Untersuchungen, W. Blumberg, Zeitschr. f. Tuberk., 1931, lix, 305.*—(*H. J. C.*)

Water Metabolism in Pulmonary Tuberculosis.—In patients with pulmonary tuberculosis water retention occurs without the formation of visible oedema. This is caused not by a disturbance of the circulatory system but by tuberculosis as an infectious disease which alters the metabolic processes. The tendency to water retention is more pronounced in patients with exudative and malignant processes.—*Zur Frage der Störungen des Wasserhaushalts bei der Lungentuberkulose, Maria Keresztes, Beitr. z. Klin. d. Tuberk., September, 1932, lxxx, 569.*—(*M. P.*)

Acid-Fast Bacteria.—The question of the occurrence of tinctorial transmutations in acid-fast and acid-sensitive organisms has long been a subject of interest. The growth and staining properties of tubercle bacilli were strikingly modified when grown on a simple nonprotein culture medium. Growth was at first less luxuriant than that usually obtained in the customary glycerinated broth, and the acid-fast characteristics of the organism were changed as a whole or in part. Cultivation of an avirulent strain of human tubercle bacillus (Novy) on the nonprotein medium, with or without glycerin, caused the disappearance of acid-fastness, usually after a number of generations. Occasionally, however, there was a reversion to type, resulting in a culture containing both acid-fast and acid-sensitive forms, with one or the other predominating. It was of interest to determine the possible effect on the staining characteristics of the addition of certain dyes to the medium. To the nutrient agar and the synthetic nonprotein medium were added, respectively, basic fuchsin, acid fuchsin, neutral fuchsin and methylene-blue in dilutions ranging from 1:100 to 1:1,000,000. The culture used as inoculum was an acid-sensitive strain of avirulent tubercle bacillus (Novy) grown for five generations on fluid synthetic nonprotein medium. In acid fuchsin no growth was observed in dilutions of 1:100 or of 1:333. The dilutions of 1:1,000 and 1:10,000 yielded organisms that were strongly acid-fast. In dilutions of 1:100,000 the organisms were strongly acid-fast in five instances, and in two a mixture of acid-fast and acid-sensitive types occurred. With basic fuchsin in dilutions of 1:100 or of 1:333 no growth was observed. The dilutions of 1:1,000 yielded acid-fast organisms five times and acid-sensitive forms once. The dilution of 1:10,000 yielded acid-fast bacteria seven times and acid-sensitive organisms once. The dilution of 1:100,000 yielded acid-fast organisms four times, acid-sensitive organisms once, and mixed forms twice. The dilution of 1:1,000,000 was tried once, and the organisms recovered were chiefly acid-sensitive. A few tests were made with neutral fuchsin and the results indicated that this dye favored the development of acid-sensitive forms. Four generations of tubercle bacilli were observed on plates containing methylene-blue. Growth was not observed in dilutions of 1:100 or 1:1,000. The dilution of 1:10,000 yielded acid-fast organisms in the first generation and retained the characteristic of acid-fastness throughout three successive transplants to the same concentration of dye. In the dilution of 1:100,000, growth contained acid-fast and acid-sensitive forms, but yielded only the acid-fast organisms in the next three generations. The first generation in a dilution of 1:1,000,000 was acid-fast and acid-sensitive, and showed only the decolorized forms in transplants to the same concentration of methylene-blue. Control plates in all series were acid-sensitive throughout. The results obtained with the virulent strains of human tubercle bacillus, H37, were quite different. Growth of this organism did not occur on agar plates containing nonprotein media, either with or without the addition of dyes. It has been generally considered necessary that a fatty substance be included in culture media for acid-fast organisms in order that they may maintain their resistance to decolorization by acids. To test this point a prolonged series of transplants incubated at temperatures of from 25° to 27°C. and 37.5°C. was made with the Novy aviru-

lent acid-sensitive strain on glycerinated hormone-broth and agar medium. The tinctorial reactions did not differ in degree from those observed in the course of transplantation on the nonprotein culture media. Marked dissimilarity in the behavior of the avirulent Novy strain of tubercle bacillus and the virulent H37 strain was observed in the experimental animal as well as *in vitro*. The intracutaneous inoculation of 0.1 mgm. of the virulent culture caused the development of a generalized tuberculosis. Intracutaneous inoculation of the same, or of a larger dose of the avirulent Novy strain caused no tubercle formation. Incidentally the inoculation of varying doses of avirulent tubercle bacilli conferred no protection against a subsequent inoculation of the virulent H37 strain. *Summary*: Certain environmental conditions, such as cultivation on nonprotein simple media, favored the loss of acid-fastness of a strain of tubercle bacilli. Such a strain was avirulent and devoid of invasiveness in guinea pigs. The avirulent, acid-sensitive strain of tubercle bacillus was not allergogenic and failed to protect animals against reinoculation with a small dose of virulent culture. It was found that lipoidal or fatty substances in culture media did not transform ordinary non-acid fast into acid-fast types. In the avirulent strain, neutral fuchsin favored the development of acid-sensitive organisms. With other dyes the selective tinctorial effects seemed to be related inversely to the bactericidal action of given dilutions. Acid-fastness was preëminently a characteristic of organisms that resisted the lethal action of concentrated solutions of the given dyes. The virulent H37 failed to grow in culture media to which the same dyes had been added. These studies appear to demonstrate the difficulty of altering the original staining and biological characteristics of a virulent strain of tubercle bacilli. Similarly, a culture that had become relatively acid-sensitive could not be readily converted into the original acid-fast type. The possibility of dissociating tubercle bacilli by means of certain specific dyestuffs might be inferred from these observations.—*Tinctorial Transmutations of Acid-Fast Micro-organisms and Virulence of Tubercle Bacilli, F. Eberson and M. A. Sweeney, J. Infect. Dis., October, 1931, xlix, 301.*—(*F. G. P.*)

Acid-fast Bacteria.—In the course of a study of acid-fast organisms, not mammalian tubercle bacilli, isolated from lesions in man, it was observed that a human tubercle-bacillus rabbit antiserum would agglutinate these organisms in almost as high titre as the homologous organisms and *vice versa*. This nonspecific factor was studied with a view to eliminating it in the preparation of a specific antiserum. As a result of these investigations it is concluded that antisera prepared in rabbits by inoculation of whole bacterial vaccines of acid-fast organisms give a strong precipitin reaction with a carbohydrate isolated from filtrates of these organisms. This carbohydrate is common to a representative group of the acid-fast organisms, as determined by precipitin tests and passive anaphylaxis in guinea pigs. It is not related to the pathogenicity or virulence of acid-fast bacilli, as it is present in the saprophyte *M. phlei*, and in avirulent strains of bovine and avian tubercle bacilli. In order to produce specific antisera for members of the acid-fast group of bacteria, it will be necessary to use antigenic fractions (protein or lipoid).—*A Carbohydrate Common to the Group of Acid-fast Organisms, Including the Tubercle Bacilli, A. Branch, Tubercle, August, 1932, xiii, 481.*—(*M. E. P.*)

Morphology of Tubercle Bacilli and Acid-fast Bacteria.—Various experiments have been made with the tubercle bacillus and apathogenic mycobacteria which are closely allied to tubercle bacilli. This predominantly botanical study had in view the question of whether the tubercle bacillus and its allies undergo a change of form or have a developmental cycle. The acid-fastness common to the tubercle bacillus and to most of the mycobacteria, and to some of the actinomyces strains, may change very much in degree. Even one given strain may change, as is apparent if it is examined at regular intervals. Young cultures are usually strongly acid-fast: in older, dried cultures this property may more or less disappear, only to reappear if the culture is brought back to a moist condition. Upon the loss of acid-fastness the bacteria show a tendency to cluster in heaps and stick together. The loss of acid-fastness of the bacteria in the living organism is not alone a sign of old age. Fermentative processes may lead to the same result. In the mycobacteria the capacity to form the acid-fast substance may be eliminated by the addition of filtrates of aspergillus and also other substances. On the other hand and in similar strains, acid-fastness may be enhanced by passage through animals. The granular forms of Much may be demonstrated in most of the mycobacteria, in many strains of actinomyces and in various other bacteria. These granular forms are apparently albuminoid substances which are not stainable by the Ziehl-Neelsen method. They are not products of degen-

eration and may be found in all age-groups. Size and form of all mycobacteria and, in a lesser degree, of all actinomyces change very much. External influences determine size and form. Small differences in the culture media may produce giant and dwarf forms; these are not permanent. The virulence of such changed forms of tubercle bacilli remains. The unchanged filamentous and branching forms are met with regularly in most mycobacteria, but not always in the tubercle bacillus, and then best when they have grown on potato-agar containing a little cane-sugar. Propagation occurs by segmentation, but in both mycobacteria and tubercle bacilli there is also a process similar to the air-spore-formation of the actinomyces in which degenerative forms are obtained. In the dark-field, a segmentation of the tubercle bacilli into 4 to 6 parts may be seen; these parts or spores which often, but not always, separate, are partly Ziehl-positive, partly Much-positive. Filtration experiments with membranous filters of varying sizes indicate that there are no filterable forms of the tubercle bacillus.—*Zur Morphologic und Entwicklung der Tuberkelbazillen, S. Seiffert, Deutsche med. Wchnschr., July 1, 1932, lviii, 1052.*—(*H. S. W.*)

Pleomorphism of Tubercle Bacilli.—Deep culture has rarely been used for growing tubercle bacilli, but the author believes it more nearly resembles conditions in the body. Nutrient-rich and nutrient-poor media, as well as liquid media prepared from serum or plasma, were utilized. A typical human strain showed in deep cultures after 2 to 4 weeks, the earliest growth appearing in pin-head-sized grains or granules without turbidity. After weeks or months there appeared sheath-like growths on the surface, resembling typical zoogloea. The forms growing in the depths are long, with many divisions, and containing dark granules, while those on the surface reveal large ovoid forms, mostly at the ends. The well-staining strong developmental forms are frequent in young cultures, and upon the addition of tuberculosis antiserum. A bovine strain under identical conditions showed the same forms. It was found that tubercle bacilli can assume variable acid-fast and non-acid-fast appearances. Although they may deviate from the typical tubercle bacilli, upon being transplanted typical forms again occur.—*Zur Veränderlichkeit des Tuberkuloseerregers in morphologischer Hinsicht, O. Kirchner, Beitr. z. Klin. d. Tuberk., 1931, lxxvii, 72.*—(*H. J. C.*)

Non-Acid-Fast Forms of Tubercle Bacilli.—A non-acid-fast strain of human tubercle bacilli obtained from strain H37 was described by the author in *Science* in 1931. This organism had the morphological characteristics of the parent strain, but its growth was more rapid, and it was chromogenic, having acquired a deep yellow-orange color. On media suitable for acid-fast growth, colonies of this organism were made up of non-acid-fast rods with a scattering of acid-fast granules and rods. On more simple media, the acid-fast elements were almost entirely absent. This organism caused tuberculosis in 3 of 5 guinea pigs. It was named the chromogenic H37 strain. The present article deals with the question of whether there exists in association with this chromogenic strain an agent which is the cause of the loss of acid-fastness and, if this agent is transferable, to use it in degrading acid-fast organisms into non-acid-fast forms. A 10 per cent water extract of the chromogenic H37 strain was made by grinding sufficient material in a mortar and filtering through N Berkefeld candles. The 6 strains of mycobacteria used in the experiment consisted of three human strains (Saranac H37, T. S., and 90), a bovine strain (B1), a smegma strain (74), and a Saranac strain of *B. phlei*. Three of these, H37, T. S., and B1 were proved to be virulent for animals. The smegma and *phlei* strains were both saprophytic, and 90 was isolated from a case of human renal tuberculosis. Stock cultures of these were grown on Petroff's egg medium and the strain to be used for an experiment was usually a ten-day growth. The procedure was to add 1 to 10 cc. of the filtered extract to a fresh transplant of this growth. The excess fluid was allowed to dry (in the incubator) before the culture was sealed. In several instances a second and a third addition of the extract were made to the culture before microscopic or macroscopic changes were noted. Each time the extract was added to the culture a control plate or tube of medium was made by the addition of the extract to it. Treated cultures were transplanted in 5 days, 2 weeks, 4 weeks and 6 weeks, with a few exceptions. The number of non-acid-fast elements which had developed prior to each transplant determined further treatment with the extract. After the development of many non-acid-fast strains, the organisms were grown on agar until cultures were obtained which appeared in smears to be entirely non-acid-fast. When this was achieved they were inoculated into media suitable for the cultivation of acid-fast growth, for example, Petroff's egg medium. The action of the extract on many cultures of the acid-fast strains mentioned was shown by typical macroscopic and microscopic changes. Growth was

accelerated and smears at five days showed many non-acid-fast elements. At two weeks the culture originally treated still showed more rapid growth than usual, but fewer non-acid-fast elements than at five days. At about the fourth to the sixth week the growth of treated cultures suddenly became fatty and moist in appearance, and the bacterial forms became definitely pleomorphic: beaded rods, granules and clumps of material without characteristic form. After these peculiar changes had occurred, non-acid-fast cultures were generally grown with ease on agar. With the *B. phlei* strain, however, it was necessary to resort to repeated transfers to eliminate the acid-fast forms. In order to test the effect of the extract on existing acid-fast organisms, tubercle bacilli (H37) were placed in 2 cc. of the extract and incubated for 96 hours. During this time smears and cultures were made at 1 hour, 24 hours, 72 hours, and 96 hours. No increase in the non-acid-fast forms was found at these intervals, but from each transplant non-acid-fast forms were grown. The sterility of the extract causing the changes was demonstrated by frequent control inoculations on various media; and autoclaved extracts had the same effect as the nonautoclaved in producing non-acid-fast forms. These facts tend to eliminate the possibility of contamination of the original or subsequent cultures with some non-acid-fast saprophyte, which might grow better on simpler media but survive on media adapted to acid-fast growth. The return of the non-acid-fast organism to an acid-fast state was a slow process. After from 4 to 8 transfers on agar, the non-acid-fast forms were transplanted to Petroff's egg media or other media suitable for the development of acid-fast growth. Of the 17 non-acid-fast growths only 4 have returned to acid-fastness thus far. These were two cultures of H37, smegma 74, and *B. phlei* and they have returned not only to acid-fastness, but the organisms are similar to the original strain, except that they still contain a number of non-acid-fast forms. This return to acid-fastness has been through the development of acid-fast granules among the non-acid-fast cocci, an agglomeration of these granules into chains which resemble somewhat the beaded acid-fast rods of old cultures, the appearance of acid-fast globules within non-acid-fast rods, and finally the development of acid-fast rods. The evidence up to the present time, namely, the similarity of morphology of the non-acid-fast growths, the regularity with which they were obtained, the transition forms in the cultures, and the fact that 4 of the non-acid-fast growths when transferred to appropriate media have returned to acid-fast forms, makes it reasonable to assume that the non-acid-fast organisms were derived from acid-fast ones and that the phenomenon occurs regularly.—*The Induced Development of Non-Acid-Fast Forms of Bacillus Tuberculosis and Other Mycobacteria, F. R. Miller, J. Exper. Med., September 1, 1932, lvi, 411.*—(*J. S. W.*)

Tubercle Bacilli of Low Virulence.—Cultures of tubercle bacilli from various types of tuberculosis, such as that of the lungs, lymph nodes, bone or skin, show a varying degree of virulence. The most vitiated strains are obtained from tuberculous lymph nodes and bone and from lupus. This is different from the diminution of virulence following continued cultivation on artificial media. By diligent search it should be possible to isolate and cultivate a tubercle bacillus from certain cases of low-grade visceral tuberculosis, such as that in the lymphoid tissue. Such strains of tubercle bacilli might be of use in protective vaccination against tuberculosis.—*Über natürlich—hypovirulente Tuberkelbazillen Stämme, B. Gosio, Deutsche med. Wchnschr., March 25, 1932, 501.*—(*H. S. W.*)

R1 Strain of Tubercle Bacillus.—A survey of 40 years' experience with the R1 attenuated strain of tubercle bacillus at Saranac Laboratory is presented. The organism was isolated by Dr. E. L. Trudeau in 1891 from the lung of a human being dying of miliary tuberculosis. All available evidence would indicate that it should be classified as an organism of human type. About two years after it was isolated, it was noticed that the cultured organisms were beginning to show diminished virulence for guinea pigs and rabbits. After six years, in ordinary doses, it did not kill rabbits and produced only very chronic lesions in guinea pigs. It was found that this strain, which would no longer produce progressive tuberculosis, would confer a partial immunity upon guinea pigs to subsequent infection with virulent tubercle bacilli. During the next twenty-five years Baldwin and Krause used it in their elucidation of the fundamental principles of immunity and hypersensitiveness in tuberculosis. Throughout the whole period of their work, the organism continued to exhibit only a limited but still definite degree of virulence for guinea pigs but none for rabbits. It was recognized that by whatever pathway an inoculation was made, an infection with the R1 bacillus persisted only in the lymph nodes. The response of the guinea pig to this bacillus has been used in experimental studies of the relationship between the various forms of pneumonoconiosis and

tuberculosis. Long-continued inhalations of pure quartz or carborundum dusts will render an R1-inhalation infection progressive and often fatal. The bacilli recovered from such lesions are not permanently altered and produce only a typical R1 localized infection in other guinea pigs. Attempts to influence the course of R1 infections by other means have failed. Reduction of the local or general resistance of guinea pigs, irritation of the lungs, and bacterial dissociation of the bacilli have failed to produce changes in the effect upon primary R1 lesions. The great practical value of this bacillus consists in its ability to produce localized nonprogressive infection accompanied by hypersensitiveness and relative immunity to reinfection.—*The R1 Strain of Tubercle Bacillus: a Record of Forty Years' Experience with an Attenuated Organism, L. U. Gardner, Tubercle, August, 1932, xiii, 504.*—(*M. E. P.*)

Ergosterol Content of Mycobacteria.—When it was found that vitamine D could be produced by the action of ultraviolet light on ergosterol, this sterol assumed importance, and a number of substances have been studied to determine their ergosterol content. Although traces of ergosterol are now known to be widely distributed in both the plant and the animal kingdom, it is chiefly found in fungi. Since ergosterol is a sterol and closely allied to the lipoid fraction of a material, it would seem that a member of the fungi characterized by a large lipoid content would be a relatively rich source of ergosterol. In a preliminary study two cultures of *Mycobacterium tuberculosis* were used, a human type and a bovine type, which had lost their pathogenicity through long cultivation on artificial media. When negative results were obtained the question arose whether the presence or absence of ergosterol in these organisms was correlated with pathogenicity. The experiments were repeated, using a larger series of cultures which included proved pathogenic strains and saprophytic species of *Mycobacterium tuberculosis*. None of the cultures contained any appreciable amount of ergosterol, although the spectrographic method of assay used is capable of detecting 0.005 per cent of ergosterol. However, two cultures, *M. leprae* and *M. phlei* apparently contained some substance that is either closely akin to ergosterol or that interferes with the absorption bands. The effect of ergosterol, both activated and unactivated, on the growth of these cultures was also studied. The growth in all tubes containing unactivated ergosterol showed marked stimulation, as compared with controls at the end of 5 days' incubation with the following exceptions: *M. tuberculosis* (*hominis*, pathogenic), which showed no growth; *M. leprae*, *M. phlei*, *M. tuberculosis* (*bovis* nonpathogenic) and *M. avium* which showed no stimulation with any amount above 1.25 mgm., while *M. leprae* showed a slight retarding effect above this. After 5 days' incubation, only three cultures, *M. berolinensis*, *M. smegmatis* and *M. tuberculosis* (*hominis*, nonpathogenic) showed stimulation of growth in tubes containing activated ergosterol, and this effect was only apparent through the concentration of 1.25 mgm. The higher concentrations of this substance, especially that of 6.25 mgm., exhibited a growth-retarding effect with all of the cultures except the nonpathogenic bovine and human strains. The added ergosterol, both activated and inactivated, caused more intensely pigmented growths in all of the cultures at all concentrations.—*Mycobacterium. Ergosterol Content of Certain Species and Effect of Ergosterol on Their Growth, P. S. Prickett and O. N. Massengale, J. Infect. Dis., October, 1931, xlix, 297.*—(*F. G. P.*)

Fate of Tubercle Bacilli in Rabbits.—By means of cultural methods it was found in a previous study that multiplication of tubercle bacilli follows intravenous inoculation of rabbits with either the human or bovine type. In the present study an attempt was made to correlate the histopathological changes in the various organs of rabbits with the number of viable bacilli contained in them, as indicated by the number of colonies isolated from each organ at various intervals following infection. The end in view was to determine if possible what reactions of the host are associated with the multiplication of the parasite and what with its destruction. Three strains of tubercle bacilli were used, a modified strain of *BCG*, one of human type, P-48A, and one of bovine type, Bovine C. The *BCG* was obtained from Dr. W. H. Park in March, 1926. At that time it caused no progressive tuberculosis in guinea pigs, but, instead of its being propagated under the conditions prescribed by Calmette, it was transplanted monthly on glycerol-agar, and when the animals were infected in the summer of 1927 it had attained considerable virulence for rabbits. However, although some rabbits died between the fourth and sixth weeks following the injection, the majority survived with almost complete resolution of their lesions by the second to the fourth month. The *BCG* was injected intravenously in doses of 1 mgm. per kg., the human and bovine strains in doses of

0.01 mgm. For the microscopic examination sections of all the organs were taken from the immediate neighborhood of those parts that were cultured. At intervals of 1 day, 1, 2, 4 and 6 weeks and 2 months equal amounts by weight of suspensions of ground lung, liver, spleen, kidney and bone-marrow in varying dilutions were seeded upon the surface of Dorset's and Petroff's egg media, both directly and after sodium-hydroxide treatment. The number of colonies of tubercle bacilli appearing upon the surface of each tube was repeatedly determined, the final reading being made after three months incubation. *Experimental observations*: The data show that although tubercle bacilli are being destroyed from the very beginning, multiplication continues to outstrip destruction until after 2 weeks, when mature epithelioid cells are formed. In any two rabbits, even at the same interval, the more effective destruction of bacilli has occurred in that animal in which epithelioid-cell and tubercle-formation are further advanced. The most rapid growth of microörganisms was found in those tissues in which the most extensive formation of new mononuclears was observed. When the parasite stops multiplying, mitosis is seen less often, and now giant cells appear at the periphery of the tubercle. Caseation and exudation into the alveoli are coincident, not with the greatest increase, but with the most rapid destruction of bacilli. Caseation is therefore not due to the mere accumulation of tubercle bacilli, though some are usually present in or about a caseous focus. Epithelioid cells persist for a considerable time after most of the bacilli within them have died, although their disintegration products may still be present. Lymphocytic infiltration and granulation tissue are seen after the greater part of the tubercle bacilli has been destroyed. *The lung after infection with tubercle bacilli of human type*: Essentially the same processes observed in the lung with the less virulent *BCG* are seen again with the human type, but from the beginning there is a more conspicuous accumulation of cells within the alveoli, and intact tubercle bacilli are more often found within these cells. Tubercle bacilli are effectively destroyed in the epithelioid cells of the interstitial tubercles. Within the alveoli the restraining influence of the epithelioid cells may be overcome and living bacilli may accumulate within these in tremendous numbers. They may then undergo extensive caseation. This caseous pneumonia is usually localized and does not become generalized. It is noteworthy that in the rabbit with the most extensive areas of caseous pneumonia tubercle bacilli are found in larger numbers, and caseation is more extensive even in the interstitial tubercles. The microörganism persists in greater numbers 6 weeks after infection than with the larger dose of *BCG* at the same time. At 2 months there is a secondary increase in the number of both stainable and viable bacilli despite the permeation of the tubercle by lymphocytes and granulation tissue. *The lung after infection with tubercle bacilli of bovine type*: The results show that the same defensive processes that have been observed in the lung with the *BCG* and human strains are overcome characteristically with the bovine bacillus. The initial reaction of the organ to the most virulent strain is more intense as expressed by a greater rush of polymorphonuclears and a more extensive multiplication of mononuclears. But also from the very beginning cellular accumulation within the alveoli, in which, as was seen before, tubercle bacilli are not readily destroyed, is more important in the bovine-type lesion, which is more diffuse and less nodular than with the human strain. These changes were accompanied by sharp increase of viable tubercle bacilli in the second week following a lag in the first week. They stopped multiplying between the second and fourth weeks, again at a time of extensive formation of epithelioid cells and of tubercles, but they persisted in the epithelioid cells collected in the alveoli. From now on the lesion is increasingly pneumonic and bacilli are found in increasingly large numbers. Again they are fewer in the interstitial tubercles, but even in these more numerous when the pneumonic areas are more extensive. The greater part of the parenchyma may become consolidated by caseous pneumonia, throughout which tubercle bacilli are found in tremendous numbers. On the other hand, they have been held in check in those rabbits in which the interstitial lesion predominates, even though the parenchyma may be extensively involved. The difference between the human and bovine types is essentially one of degree. Usually with the former the process is chiefly interstitial; it is circumscribed and never becomes generalized. The pneumonic areas when present are localized and tend to become surrounded by a productive process. Caseation is limited; the regeneration of mononuclears soon ceases; giant cells appear at the periphery; lymphocytes and granulation tissue permeate the tubercle; in some instances after the lesion has persisted 2 months, softening and cavity-formation overtake the isolated residual tuberculous foci. With the bovine infection, the lesion is chiefly of a mixed pneumonic and productive character;

it spreads over almost the entire lung; caseation appears early and becomes massive. The zone of intact epithelioid cells is slight and regeneration of mononuclears goes on unabated. Giant cells do not form at the periphery of the interstitial tubercles, though they have been seen in the pneumonic lesion. Lymphocytes do not permeate the tubercle to any considerable extent and the granulation tissue appears as islands between the multiple foci of caseation. The caseous pneumonia is widespread throughout the lung. Softening and caseation were not noted within the course of the experiment. Associated with these differences is the effective destruction of the human bacillus within the interstitial lesions. With the bovine on the other hand the bacilli are held in check but not destroyed in these lesions, whereas in the pneumonic areas they accumulate in far greater numbers than the bacilli of the human type. Essentially the same correlations between histological changes and the fate of tubercle bacilli as found in the lungs were seen in the other organs involved. The culture method is more efficient than the histological method in indicating quantitative relations. Tubercle bacilli were not perceived in the organs of rabbits one day after infection with the modified *BCG*, although as many as 1,500 colonies were isolated from one of them. The culture method also gives evidence concerning the number of living tubercle bacilli in the tissue. The accumulation of acid-fast particles is greater in the Kupffer cells in the liver, in the macrophages of the spleen and in the reticular cells of the bone-marrow than within the mononuclears of the lung. Acid-fast particles are more prominent with the bovine than with the human bacillus or the *BCG*, the microörganism that is destroyed with the greatest difficulty, thus leaving more incompletely digested bacillary debris at a given time within the cells. Tubercle bacilli never accumulate in such large numbers in the mononuclears of the liver as they do in the lung. Though at first the tubercles in the liver may be more numerous than those in the lung they never attain the same size. The formation of new mononuclears is restricted and Langhans giant cells appear very early. The liver tubercles are resorbed early even with the bovine infection. Similar differences were observed between the splenic pulp and corpuscles. In the former the accumulation of acid-fast particles was much greater and the tubercles developed earlier. Mitosis of mononuclears was less frequent and giant cells appeared earlier. Tubercle bacilli, always intracellular, disappeared from the tubercles in the pulp sooner than from those in the corpuscles, and the tubercles themselves first disappeared from the pulp. These observations indicate that the mononuclears of different organs, or even of the same organ, as in the different parts of the spleen, have a different capacity to destroy the tubercle bacillus, and that the transformation of the mononuclear into the mature epithelioid cell follows its destruction of the tubercle bacilli. In the lung the more virulent types of bacillus are destroyed within the epithelioid cells of interstitial tubercles but persist in foci of tuberculous pneumonia. But with both strains tubercle bacilli in large numbers may accumulate within the epithelioid cells lying free in the alveoli. There is apparently some factor acting in the alveoli that favors the growth of the parasite. This factor may be the greater oxygen tension of the alveolar air. An intense multiplication of mononuclears is associated with the growth of the tubercle bacillus and their transformation into mature epithelioid cells is associated with its destruction. The Langhans giant cells have often been considered an indication of the chronicity of the pathological process. It would appear that they are formed from existing epithelioid cells when the multiplication of the bacillus has ceased and the stimulus for the formation of new cells has decreased or stopped. Lymphocytes and granulation tissue do not cause the destruction of tubercle bacilli, these being destroyed in their absence. Immediately after infection, accumulation of the less virulent types of tubercle bacilli in the tissues does not cause caseation, and the more virulent bovine bacillus produces this change only in the lung. Later caseation occurs in the presence of a small number of bacilli, and must be thought of as due, in part at least, to sensitization.—*The Correlation between the Histological Changes and the Fate of Living Tubercle Bacilli in the Organs of Tuberculous Rabbits*, *M. B. Lurie*, *J. Exper. Med.*, *January*, *1932*, *lv*, *31.*—(*F. G. P.*)

Toxicity of Iodine for Tubercle Bacillus.—The avidity with which iodine is taken up by the membrane and the granules of the tubercle bacillus suggests that this substance should have a strong germicidal action against this organism. The literature dealing with the subject is scanty and the results obtained are variable. The strains of the tubercle bacillus used in the present investigation were the human strain, H37, and a bovine strain, BR. The organisms were suspended in sterile water and filtered through a sterile Whatman paper. One cubic centimetre of the filtrate was then

measured out and mixed with a known volume of sterile skimmed milk. Of this mixture, 0.01 cc. was spread over an area of 1 square cm., allowed to dry, fixed over the flame, stained by the Ziehl-Neelsen method and later counted. Five more cubic centimetres of the filtrate was measured into a large, sterile pyrex test-tube which had been warmed to 37.5 C., and 20 cc. of iodine solution (1:5000) at the same temperature was poured quickly over the bacterial suspension and mixed. At proper intervals, injections were made subcutaneously into guinea pigs. In each of three experiments a series of test-tubes containing different amounts of a 1 per cent iodine soluton in 3 per cent aqueous potassium iodide was placed in a water bath at 37.5°C. In each tube the volume was made up to 1 cc. with sterile physiological salt solution. Each received 1 cc. of a suspension of tubercle bacilli at the same temperature. At definite intervals, a loopful was taken from each tube, mixed in a sterile petri dish with a droplet of a previously boiled, tenth-normal sodium-hydroxide solution, to neutralize the iodine, and planted on a slant of Petroff's glycerin-egg medium. A loopful of the original suspension was transferred to the same medium, with and without passage through alkali, for controls. The results of the present experiments show that iodine is toxic to the tubercle-bacillus cells, for these cells are unable to grow on slants after thirty minutes contact with as high a dilution of iodine as $1:10^4$, or to infect guinea pigs after an exposure of fifteen minutes to iodine in the dilution of $1:5 \times 10^3$. These results are contrary to the report of DeWitt and Sherman, that compound solution of iodine is not able consistently to kill the tubercle bacillus cells even after an exposure of twenty-four hours. These authors used cell-clumps in their test-tube experiments, and dried cells in their experiments on animals. When a clump of cells is placed in an iodine solution, the iodine reacts with the lipoids of the cells on the outside, and this slows down its diffusion to the interior of the clump. On the other hand, it is a fact that the cells become less permeable on drying.—*The Toxicity of Iodine for the Cells of Mycobacterium Tuberculosis, G. Knoysi, J. Infect. Dis., March, 1932, l, 255.*—(*F. G. P.*)

Anomalous Behavior of Tubercle Bacillus.—In a review of a monograph by the present authors, Wilson included the statement that, on the basis of the thesis put forward by them that the bacterial cell behaves as an equilibrated system of two classes of ampholytes, one more acidic in nature than the other, "no explanation is afforded for the anomaly that the tubercle bacillus, though Gram-positive, has yet a high resistance to the bacteriostatic effect of gentian violet." Experimenters have noted considerable divergences among strains of tubercle bacilli in their staining properties and their dye sensitiveness. While some of these divergences may be due to the difficulty in measuring the inoculum and in preventing the transfer of clumps, this cannot be the entire explanation. Variability both in staining and resistance, while found to some extent in many if not all organisms, is so striking in the tubercle bacillus as to indicate factors at work which are either absent or much less effective in many bacterial species. All of the strains of *B. tuberculosis* studied have, in a very marked form, the ability to decolorize gentian violet if the dye concentration will permit growth at all. This decolorization is not merely a removal of the dye from the medium by adsorption on the bacterial mass. The tubercle bacilli, taken from these decolorized media, show no evidence of staining when examined microscopically. This organism seems to have a decomposing action on the dye, changing it to a form which is found to be noninhibitive to *B. subtilis* even at high concentrations. The Gram character of *B. tuberculosis* is dependent on the acid-fastness. Removal of the material which causes acid-fastness yields a product which is no longer acid-fast.—*The Anomalous Behavior of the Tubercle Bacillus as a Gram Positive Organism with High Resistance to Gentian Violet, E. W. Stearn and A. E. Stearn, J. Bact., May, 1932, xxiii, 399.*—(*F. G. P.*)

Bacterial Counts of Suspension of Tubercle Bacilli.—The most dependable method for the counting of bacilli in suspensions of tubercle bacilli is the one published by Lange, in which a suspension prepared in a slightly alkaline solution is seeded on egg media. In order to obtain reliable counts it is advised that three series of six tubes each be used and that the suspensions are diluted to the point that in one series 30–50 and in other series 3–5 colonies are obtained.—*Erfahrungen mit der Bestimmung der Keimzahl in Tuberkelbacillenaufschwemmungen, O. Kirchner and B.D. Li, Beitr. z. Klin. d. Tuberk., August, 1932, lxxx, 454.*—(*M. P.*)

Implantation of Capsules with Tubercle Bacilli in Animals.—Capsules of wood, filled with tubercle bacilli were implanted in the peritoneal cavities of rabbits and sheep. It was found that tubercle bacilli never escaped from these capsules,

since tuberculous lesions were never found in the animals, even after experiments of long duration. This would indicate that the tubercle bacillus has no filterable elements. The tubercle bacilli in the capsule become disintegrated and lose their invasive power. The serum of the experimental animals yields complement-fixing antibodies and some protective properties against infection with tubercle bacilli. The animals become allergic to tuberculin without developing tuberculous foci.—*Tierexperimente zum Tuberkuloseproblem, K. Preisich, Beitr. z. Klin. d. Tuberk., August, 1932, lxxx, 496.*—*(M. P.)*

Tuberculosis from Intratracheal Injections in Rabbits.—The aërogenous infection of animals with the tubercle bacillus is of interest for two reasons: first, because of the scantiness of experimental studies, and second in connection with recent views of the pulmonary structure. The author has previously shown that the inflammatory reaction in pathological conditions of the lung originates *in situ* from the cells without and within the alveolar septa and also from the peribronchial and perivascular cells. The cells commonly designated as "alveolar epithelium" are to all appearances not epithelial, but of mesenchymal origin. The present study with the tubercle bacillus was undertaken in order to find new evidences pertaining to this theory. The experiments were conducted on full-grown rabbits. A six-weeks-old culture of bovine tubercle bacilli grown on glycerin-agar was used. In each case 0.5 mgm. of the bacilli emulsified in 1 cc. of physiological salt solution was injected into the lung by way of the trachea. The animals were killed by air embolus at intervals of from one minute to several weeks after the intratracheal injections. Sections of the lungs were made, stained and examined. *Findings:* When the tubercle bacilli reached the air-sac the reaction caused by them was almost instantaneous, for within one minute after the aërogenous infection there occurred a marked proliferation, as well as morphological changes, of cells lying in and on the septum. These cells, which are normally barely visible, giving to the air-sac a "naked" appearance, revealed characteristics that are proper to fixed or to wandering macrophages (monocytes and clasmatocytes). A number of these cells lay free in the alveolar lumen, showing phagocytosis of the tubercle bacilli; the bacilli were also found in the cytoplasm of some of the enlarged sessile cells. Cells in mitosis and amitosis were seen here and there, and within the next few minutes primitive tubercles made up of these (epithelial) cells transformed into epithelioid cells were seen scattered over the section. At this period, too, outlines of giant cells could be made out budding from the septa, resulting from the "agglutination" of individual cells and also from the nuclear division of cells. As a rule the newly formed cells proliferated inside the lumen of the alveolus, causing a "parenchymatous alveolitis." This process ordinarily involved a group of several alveoli, of which the smaller were completely occluded by the epithelioid cells, while the larger were occupied only in part. In addition to the prompt morphological changes and the rapid proliferation, the sessile and the free cells also displayed an instantaneous phagocytosis of the acid-fast bacilli. The endothelium of the capillaries of the septa showed no particular changes. Their lumina contained a moderate number of red cells, but no mononuclear leucocytic elements. These findings show, first, that in tuberculosis of the lung the cells that primarily respond to tubercle bacilli are not called from outside sources, but are produced *in situ*. In the second place they show that the source of these cells is not the endothelium of the pulmonary capillaries (endothelial leucocytes), but cells that "line" the walls of the air sacs, as well as those that are normally present in the loose connective tissue of the septa. Particularly instructive, too, were the pictures in close proximity to the primitive tubercles. It appeared that not only did the morbid process advance by proliferation of its own elements, but at its borders it also "converted" the adjacent cells. Here, also, the cells while still resting on the septa, had taken on new morphological aspects, thus giving them the appearance of large monocytes. In proximity to the primitive tubercle these cells could be seen to "stream" singly or in groups to join and to enforce the already formed "miliary granule." Thus, when the agglomerated tubercle had reached considerable dimensions, and its centre had begun to degenerate (caseation), its periphery was made up of new elements that resulted not only from proliferation of its own cells, but also from the mobilization of those resting in the adjacent areas. The wall of the air-sac representing no more than a "spider's web" of loose connective tissue reënforced with a few elastic fibres, also contains interlacing bundles of innumerable capillaries and a sparse number of scattered cells. These cells, when sectioned at various angles, appear either as elongated cells lying "astride" on the capillary as do the adventitial cells of Marchand, or as round lymphocytoid cells having somewhat bean-shaped nuclei, but coarse, darkly

stained, chromatin particles. The resemblance of these cells to lymphocytes is remarkable. In the experiments presented here, the true pulmonary lymphocytes did not seem to play a part in the production of macrophages. Apparently the cells found in the walls of the septa are not other than undifferentiated mesenchymal cells, which here, as indeed elsewhere in the organism, readily serve as a source of the wandering macrophage. They are probably adventitial cells. Whatever the nomenclature of these cells may be, they are, it is reasonably certain, of mesenchymal origin and act as such; they store dyes and oils; they avidly phagocytose microörganisms; they play a rôle in the extrahepatic formation of bile; they likewise participate in the formation of the tubercle. Attention is called to the "defensive" rôle of the lung. Under the conditions of the experiment discussed above, rabbits that have been infected with bovine tubercle bacilli by way of the trachea survive three or more times longer than those that have received a similar dose of bacilli by way of the blood-stream. In many animals that have been injected by way of the trachea the acid-fast microörganisms apparently remain "immured" in the lung "for life" as they cannot be detected outside these organs. The lungs are not only a "sieve" or a "filter" but are a virtual "fortress," which retains and destroys not only foreign elements, but pathogenic microörganisms. It is true that haematogenous elements (monocytes and also lymphocytes) contribute to the formation of the tuberculous granulation tissue of the lung. However, the afflux of these cells from outside sources occurs at a later date and obviously does not play an important rôle when infection occurs by way of the respiratory tract. It is of interest that granulocytes, which are found in numbers in the lungs of rabbits infected with tubercle bacilli by way of the blood-stream, are insignificant in aërogenous infections. They reach the lung from the periphery. That the lungs react as do mesenchymal organs and that the essential source of the reactive elements is not the blood or the endothelium of the capillaries, but the cells lining the air-sacs as well as those found within the septa is apparently not contested. What is in dispute to-day is whether the alveolar epithelium is derived from the mesoderm (mesenchymal) or whether it is epithelial in character. The author accepts the theory of the mesenchymal nature of the alveolar epithelium. Reproductions of microphotographs accompany the article.—*The Infection of Rabbits with the Tubercle Bacillus by Way of the Trachea. Studies on the Defensive and Metabolic Apparatus of the Lungs, B. M. Fried, Arch. Path., November, 1931, xii, 689.*—(*J. S. W.*)

Inflammation and Immunity.—In the usual textbook description of the inflammatory reaction, except for an occasional reference to the importance of the accumulation of serum at the site of inflammation, the predominant rôle of the leucocytes completely overshadows the activities of all the other elements. Recently it has been shown that the inflammatory reaction plays a definite rôle in localizing the irritating factor, or at least in limiting its dissemination, whether the irritant be bacterial or chemical. This capacity, which may be appropriately termed *fixation*, contributes a further significance to inflammation in relation to immunity. By delaying the dissemination of obnoxious substances into the circulating blood, the nonspecific as well as the specific inflammatory reaction locally protects the organism as a whole at the expense of local injury. Recent investigations by the author have demonstrated that fixation at the site of inflammation is due to mechanical obstruction caused by a network of fibrin and to occlusion of lymphatic vessels. The fixation is the initial stage in the development of the inflammatory reaction, even preceding the migration of the leucocytes. This is doubtless due to injury of the capillaries causing an increase in the permeability of their walls, which results in an outpouring of fibrinogen into the tissue-spaces. Pathologists are gradually reverting to the concept of Cohnheim, namely, that in inflammation the primary injury is to the wall of the capillaries. The purpose of this review is to describe the evidences that recently have accumulated which show that, with the initial increase in the permeability of the capillaries and the passage of plasma constituents into tissue-spaces, the inflammatory reaction is capable of rapidly circumscribing the irritant, thus allowing a definite interval of time for the leucocytes to assemble for phagocytosis. Fixation, as the initial phase of the inflammatory reaction, therefore becomes of distinct importance in the mechanism of immunity. *1: Fixation of bacteria by the nonspecific inflammatory reaction:* The dissemination of foreign substances from the site of injection into the lymphatics and into the blood-stream is rapid. Carmine, when injected into the peritoneal cavity, reaches the anterior mediastinal lymph nodes very rapidly (Muscatello); bacteria injected into the knee-joints of rabbits have been found in the inguinal crural and lumbar lymph nodes in from five to ten minutes (N[illegible]). Ty-

phoid bacilli have been found in great numbers in the blood-stream within a few minutes after their injection into the peritoneal cavity (Buxton). The absorption of bacteria from this cavity takes place through the lymphatic vessels. Does the inflammatory reaction retard the dissemination of microörganisms from the site of inflammation? Issayeff has shown that a peritonitis induced by a variety of sterile irritants temporarily increases resistance to subsequent intraperitoneal injections of bacteria. Pawlowsky found that an acute inflammation of the knee-joint produced by the injection of sterile irritants inhibited or prevented the dissemination of staphylococci when injected into the joint. Other investigators have confirmed these studies. Some authors believe that in inflammation the large mononuclear phagocytes are responsible for protection, and that polymorphonuclear leucocytes are relatively inactive in increasing the resistance. However, the increased resistance to virulent bacteria brought about by previously "preparing" a cavity with a sterile irritant is only evident when the inflammatory reaction has been going on in the cavity for from forty-eight to seventy-two hours. Local fixation of substances at the site of inflammation can be demonstrated (see below) a relatively short time (thirty minutes) after the introduction of the inflammatory irritant. *2: Fixation of bacteria by the specific inflammatory reaction:* Koch first described the immune reaction that takes place when a tuberculous animal is reinoculated with either living or dead tubercle bacilli. Pirquet called the altered response of tuberculous tissue to reinfection *allergy*. According to Opie, the tuberculin reaction, which may be regarded as a manifestation of the Koch phenomenon by means of tuberculoprotein, is histologically an acute inflammation. During the first forty-eight hours there is an abundant accumulation of serum and polymorphonuclear leucocytes. Mononuclear cells finally displace the polymorphonuclear leucocytes. Krause has suggested that specific immunity in tuberculosis might be a function of allergy in that the inflammation accompanying the latter prevents the spread of reinfecting bacilli. Willis observed that in reinfected guinea pigs the spread of tubercle bacilli from the site of inoculation was retarded, whereas in normal animals the organisms passed readily to the regional lymph nodes. Both Krause and Willis believe that "specific tuberculo-immunity occurs through a fixation of germs that results from the operation of the allergic reaction. An almost immediate inflammatory outpouring hems in the bacilli more or less effectively and thus delays or prevents their spread, which is so facile and rapid in the nontuberculous animal." Rich and McCordock attacked the hypothesis of Krause and Willis, pointing out that, although it was assumed that inflammation *per se* can limit the immediate spread of microörganisms, there was not the slightest proof of this. They held that the inoculation results in the experiments of Krause and Willis might be interpreted on the basis of the well-established fact that there occurs a much greater destruction of bacilli in immune animals than in normal ones. However, Willis demonstrated that the regional lymph nodes in immune animals were infected at the end of two weeks after cutaneous inoculation. If the apparent retardation of bacilli at the site of inoculation was really due to their destruction *in situ* by a subtle immunological mechanism, their appearance after a latent interval of two weeks in the regional lymph nodes would be difficult to explain. Menkin has recently demonstrated that an acute inflammatory reaction caused by a nonspecific irritant prevented for at least several hours the dissemination of *B. prodigiosus* to the tributary lymph nodes. The view that resistance may at times be independent of allergy and thus referable entirely to an immunological mechanism is not questioned. Zinsser and his collaborators have expressed the view that "the fundamental biological significance of bacterial allergy is an increased specific adjustment of the tissues for response to the stimulus of infection, and a consequently enhanced capacity for the rapid mobilization of a protective mechanism." The fundamental point, however, is this: When an allergic inflammation develops as a result of reinfection, does the inflammatory reaction play a definite rôle in the mechanism of immunity by retarding the dissemination of bacteria? Affirmative evidence has been cited and is to follow. *3: Fixation of foreign proteins by the specific inflammatory reaction:* Extensive studies by Opie demonstrated that a foreign protein injected into the skin of a sensitized animal is fixed at the site of injection. This fixation is accompanied by an acute inflammatory reaction (Arthus phenomenon). When a foreign protein is injected into a normal rabbit, it is demonstrable by the precipitin reaction in the blood of the animal for a period of from seven to nine days. Opie has shown that with continued immunization, injected proteins exhibit a decreasing tendency to find their way into the blood. After the sixth or seventh injection well-immunized animals reveal no trace of the proteins in the circulating blood. By excising the cutaneous

site of the injection and estimating the amount of foreign protein present, Opie demonstrated that in the immunized animal the antigen was fixed at the site of injection, and this prevented its dissemination throughout the body. He was of the opinion that specific precipitation had a part in this fixation. The precipitate formed by the contact of antigen and antibody has a strong attraction for polymorphonuclear leucocytes of the blood, and is doubtless digested and destroyed by these cells. Menkin has demonstrated that a nonspecific acute inflammatory reaction is capable of arresting a foreign protein at the site of inoculation. For this reason it is probable that the fixation of antigen in the Arthus phenomenon is largely due to the acute inflammatory reaction brought about by the contact of antigen and antibody in the tissues. *4: Accumulation and fixation of foreign substances at the site of inflammation:* In an inflamed area the normal functional equilibrium between cells, intercellular fluids, and blood is doubtless profoundly modified. The permeability of the capillaries, the rate of the blood-flow, and the balance of the body fluids are affected. The increase in the permeability of the capillaries is shown by the local oedema. The normal equilibrium of the filtration through the wall of the capillary, however, is doubtless modified in inflammation by the passage of proteins from the plasma into the tissue-spaces. The accumulation of plasma proteins in these spaces may modify and retard the flow of lymph into the different lymphatic vessels. An inflamed area can be considered as shunted off from the rest of the organism. Menkin studied the behavior of trypan-blue when injected into the subcutaneous tissue of the foreleg of a rabbit. In normal tissue the dye stains the tributary afferent lymphatic vessels, the lymph node, and the efferent lymph vessel, within from twenty to thirty minutes. If, on the other hand, the dye is injected into a similar area in which an inflammatory reaction has been produced some time before by a sterile irritant, the dye does not appear in the lymphatics and is evidently retained in the inflamed area. The fixation of the dye has been observed when the dye is injected as early as thirty minutes after the injection of the irritant. Since trypan-blue tends to remain *in situ* when injected directly into the inflamed area an attempt was made to determine whether dye injected into the blood-stream would accumulate at the site of the inflammation. The trypan-blue was found to enter rapidly the site of inflammation, staining the tissue deeply, and it did not readily drain away through the tributary lymphatic vessels. The longer the interval of time between the injection of the irritant and the dye, the more complete was its retention, less of the dye diffusing to the regional lymph node. Thus there was not only a rapid accumulation, but also a fixation of dye from the blood-stream in the inflamed area. The accumulation of the dye is doubtless the result of the increased permeability of the capillaries which is part of the inflammatory reaction. When injected intravenously trypan-blue was found to stain deeply an area of injury (burn) in the cornea of a rabbit whereas the corresponding normal area in the other eye showed no blue or only a minute trace of it. Metals, like the dyes, were found to be fixed *in situ* by the inflammatory reaction. Iron was selected because of the ease of detecting this metal in tissues by the prussian-blue reaction. When injected into the normal peritoneal cavities of rabbits it rapidly accumulated in the retrosternal lymph nodes, but it failed to reach these lymph nodes when injected into inflamed peritoneal cavities. When injected into the circulating blood about three times more metal accumulated in inflamed than in normal areas of the skin. Menkin also has demonstrated by qualitative and quantitative methods that daily intravenous injections of ferric chloride in tuberculous rabbits resulted in an accumulation of iron in tuberculous foci. Whereas in ordinary inflamed areas the iron salts had been found to accumulate at the periphery and not in the central portion (where the circulation is relatively inactive), these tuberculous foci, on the contrary, revealed the metal in the caseous centres. It is to be noted in this connection that various dyes that cannot penetrate living cells are able to stain dead or dying cells. Experiments were undertaken by Menkin to determine whether a readily identifiable foreign protein injected into an area of inflammation would also be retained *in situ* by the inflammatory reaction. It was found that horse-serum, injected into an inflamed peritoneal cavity (rabbit), penetrated into the blood-stream less rapidly than when introduced into the normal cavity. The presence of the foreign protein was determined by the precipitin reaction on blood samples removed at varying intervals from the heart. Furthermore it was noted that, when a foreign protein was injected into a cutaneous inflammatory area, it was held *in situ* for a longer period than when injected into an inflamed peritoneal cavity. Thus the foreign proteins, such as are found in horse-serum, as well as trypan-blue and iron, are held fixed by the inflammatory reaction. Horse-serum, when injected into the circulating blood-stream,

was also found to accumulate in inflamed areas to a greater extent than in normal tissues. It is possible that the phenomenon of focal reaction in tuberculosis may be explained by these observations. When tuberculin is introduced into the blood-stream of an animal with a tuberculous lesion, an intense inflammatory reaction may develop in the lesion. The mechanism of this focal reaction in tuberculosis is not understood. It is conceivable, in view of the above experiments with horse-serum, that in a like manner tuberculoprotein from the blood stream may accumulate in the inflamed tuberculous lesion and by its presence there induce a local inflammatory reaction. Further studies were undertaken to determine the behavior of particulate matter and of bacteria at the site of inflammation when injected intravenously or directly into an inflamed area. Experiments have shown that the failure of bacterial dissemination from the site of inflammation was due to the fixation of the microörganisms. Also it has been found that dilute india ink or particles of graphite failed to disseminate when injected into an inflamed peritoneal cavity. The attempt was next made to determine whether india ink, injected into the circulating blood, would rapidly enter an inflamed area. It did not, but it is well known in this connection that india ink adheres to the endothelial lining of capillaries. Graphite ink has not this disadvantage. When injected into the blood-stream of a rabbit, graphite particles were found to collect readily in an inflammatory reaction previously induced in the skin of the abdomen. Histological section of such an area showed many polymorphonuclear leucocytes loaded with graphite. The question arose as to whether particles of graphite injected into the circulating blood are brought to the site of inflammation by leucocytes (diapedesis) or whether the permeability of the capillary is also sufficiently increased to allow some of these relatively large particles to pass through the endothelial wall. To settle this point, histological examination was made of areas of skin in which the duration of inflammation was only about six hours. Careful studies revealed many capillaries with unattached particles of graphite within their lumina. Some of these particles were in the process of passing into the extracapillary spaces. Only occasionally in such regions could the particles be seen within the polymorphonuclear leucocytes. Since no such evidence was obtained in sections of normal areas, the observations indicate that with inflammation the walls of the capillaries became permeable to particulate matter. Experiments indicate that bacteria are fixed by the inflammatory reaction like particles of carbon. It is also known that injury may determine the localization in the damaged tissue of bacteria or of ultrafiltrable organisms present in the circulating blood. Some observers believe that bacteria may become localized in places of lowered resistance. Menkin undertook experiments to determine whether bacteria (*B. prodigiosus* and *B. pyocyaneus*) would accumulate rapidly at the site of inflammation when injected into the blood-stream. Rabbits were used and an area of inflammation was induced by injecting 0.5 cc. of concentrated broth into the skin of the abdomen. After a short time, the bacteria were injected into the blood-stream. Later by cultural methods, the number of intravenously injected bacteria accumulating at the site of inflammation was found to be distinctly greater than that in an area of normal skin. The frequent localization of bacteria from the blood-stream in a *locus minoris resistentiae* occurring after preliminary trauma or other injury of tissue may be explained in terms of increased permeability of the capillaries, with resulting accumulation and fixation of bacteria at the point of injury. *5: The mechanism of fixation by the inflammatory reaction:* The leucocytes are probably not a very significant factor in the mechanism of fixation. Phagocytosed particles were never found in the leucocytes of the inflamed area at a time when fixation of foreign substances was demonstrable by examination of the tributary lymphatics. The occurrence of fixation (trypan-blue) at an early stage of the inflammatory reaction when there are yet relatively few leucocytes present seems to point to some other agent responsible for fixation. A factor that might explain fixation is mechanical obstruction. It is conceivable that a network of fibrin and thrombosed lymphatics at the site of inflammation might arrest the passage of particulate matter injected into the inflamed area. The dissemination of fluids would probably also be retarded by mechanical obstruction of this kind, though probably not as effectively as that of solid particles, which would be more readily caught in a fibrinous network. Experiments to determine whether the inflammatory exudate in itself possesses some property that might facilitate the fixing of foreign substances in the inflamed area resulted as follows: When ferric chloride was added directly to the inflammatory exudate, heavy precipitation occurred. When horse-serum was added to the exudate and incubated at 37°C. for a short time, coagulation took place. However, when trypan-blue was added to an inflammatory exudate,

no effect was noticed, yet the vital dye was shown to be definitely held *in situ*. The reaction of fixation must be due primarily to some mechanism other than precipitation or coagulation, although these phenomena may act as secondary factors. Sections were made of the inflamed tissues of rabbits in experiments in which either trypan-blue or ferric chloride had been shown to be fixed *in situ* by the inflammatory process. Histologically there was little evidence of phagocytosed particles of trypan-blue or of iron within the leucocytes at a time when retention of these substances at the site of inflammation was clearly demonstrable. A mesh of fibrin was usually found at the periphery of a central zone of dense leucocytic infiltration. In the same region many of the lymphatic vessels were found to be thrombosed. The thrombus is characterized by numerous leucocytes within a delicate fibrinous reticulum. The fact that there are many occluded lymphatics and a dense net-work of fibrinous strands within the tissues that are distended with oedema at the site of inflammation supports the view that foreign substances, especially solid particles, such as precipitated iron salts, can disseminate only with difficulty from the inflamed area through the regional lymphatics. If such factors prevent the free passage of substances from the site of inflammation, it follows that similar substances injected at the periphery of the inflamed area should be prevented by the same obstruction from entering it. To test this hypothesis trypan-blue was injected in from four to six places at the periphery of an inflamed area in the skin of a rabbit. In this way the inflamed area became circumscribed by a blue band. In an area of normal skin similar injections of dye were made to serve as a control. Several hours later the inflamed area within the original circumscribed blue band showed no trace of dye, whereas the area of normal skin was diffusely blue. The dye had evidently failed to penetrate into the site of inflammation, when injected at its periphery, owing to the presence of thrombosed lymphatics and of a fine net-work of fibrin in the tissue spaces. Similar results were obtained with bacteria. *Conclusions:* The experiments described present proof that various foreign substances injected into the site of inflammation are fixed *in situ* and fail to drain readily into the tributary lymphatic vessels. These same substances injected intravenously accumulate rapidly in inflamed areas. This accumulation is partly associated with increased permeability of the capillaries, but is also the result of the inability of these substances to escape from the site of inflammation, owing to the presence of a fine net-work of fibrin and of thrombosed lymphatics. The mechanism of fixation takes place extremely early in the inflammatory process. The earliest change in inflammation is an increase in the permeability of the capillaries which permits the passage of fibrinogen from the plasma into the tissue-spaces. The rapid formation of a net-work of fibrin and of thrombi in lymphatics at the site of inflammation circumscribes the irritating substance and thus prevents its passage into the blood-stream. This allows a definite interval of time for the leucocytes to assemble for phagocytosis. The initial fixation of bacteria or other injurious substances at the site of inflammation thus becomes a protective mechanism and plays a definite rôle in immunity.—*An Aspect of Inflammation in Relation to Immunity. A General Review (and Report of Experiments), V. Menkin, Arch. Path., November, 1931, xii, 803.—(J. S. W.)*

Koch Phenomenon and Immunity.—The significance of this phenomenon for the mechanism of immunity against tuberculosis was revealed by later investigators (1906–1910), and since then it has been the foundation upon which modern teaching of the prophylaxis against tuberculosis is built. Neither animals nor human beings react in the same way to reinfection as they do to first infection. Koch stated that upon reinoculation, animals developed inflammation and necrosis at the site, and that dead tubercle bacilli or tuberculin led to a quite similar manifestation. This is a heightened sensitiveness to the microörganism and also to the very complicated proteins and phosphatides of the tubercle bacillus. The hypersensitiveness of the tuberculous individual, which we have known since Pirquet, suggested the name tuberculin *allergy*, which is one of the manifestations of the Koch phenomenon. Hypersensitiveness proves, on the one hand, the existence of tuberculous infection, and, on the other, indicates an immunity to superinfection. In tuberculosis there is immunity or resistance to superinfection only as long as the body harbors tubercle bacilli. Similar circumstances exist in the case of syphilis and brucellosis. The resistance to superinfection diminishes gradually and disappears when the last immunizing bacilli are eliminated from the organism. The healed organism may again be infected. Only a slight infection caused by a small number of bacilli or by slightly virulent bacilli create the condition of immunity. The high percentage of positive tuberculin reactions shows that bacillary parasitism is not to be confused with tuber-

culous infection or tuberculous disease, which latter shows a tendency to spread and which causes the development of cellular and humoral defense reactions that lead to tubercle-formation and intolerance to superinfection. The simple parasitism does not lead to specific changes but to occult, stationary changes only. There is a symbiosis between the bacilli and their host, the lymphoid system. This symbiosis, if not destroyed by superinfection, creates the protecting preimmunization state. This state is hardly ever observed in guinea pigs and rabbits, as they are inoculated with formidable doses of tubercle bacilli; if these animals are reinoculated 15 to 20 days after the first infection the tolerance against superinfection is shown in the form of the Koch phenomenon, but the animals die. This picture is different in the more resistant cattle and human beings. If the first infection is made with few or with avirulent bacilli, such as the *BCG*, the organism is able to resist a natural superinfection, such as happens when such people are in contact with tuberculous individuals. This resistance is not shown by the conspicuous Koch phenomenon but either by immediate fixation of the superinfecting bacilli in a lymphatic organ or by elimination of those bacilli with the normal excreta (bile, intestinal contents, mammary secretion, etc.). This presupposes a special immunity of the preimmunizing disease created by a slight infection. To the Koch phenomenon we owe the knowledge of tuberculin allergy; that it is the expression either of tuberculous disease, latent bacillary infection or an immunity against tuberculosis. We know to-day that protection against this disease is possible only if each human being takes a few living bacilli promptly and as soon as possible after birth in a natural or an artificial way. These cause in his body an occult stationary, harmless, allergizing infection. Thus Koch prepared the way for *BCG* vaccination of newborns or nonallergic individuals.—*Das "Koch Phänomen" und die Immunität gegen Tuberkulose, A. Calmette, Deutsche med. Wchnschr., March 25, 1932, 499.*—(*H. S. W.*)

Pirquet Reaction and Immunity to Tuberculosis.—It has been observed that the Pirquet reaction and immunity against tuberculous infection are reciprocally related. Among 454 persons with positive Pirquet reactions, 12 cases of tuberculosis developed, or 2.6 per cent, while among 253 subjects with negative reactions there were 75 cases of tuberculosis, or 29.6 per cent. A graph is given which reveals that the results for each year have not been uniform among the persons vaccinated with *BCG*. These subjects fall into two groups: first, those vaccinated during the years 1927, 1928, and 1929, and, second, those vaccinated during 1930 and 1931. In the first group, 6 cases of tuberculosis occurred among 137 vaccinated persons. The period of observation extended from two years to four years and a half. In the second group, there were 14 cases of tuberculosis among 70 vaccinated persons, the longest period of observation being one year and a half. A total of 207 vaccinations were made. Forty-three of these were eliminated from the final appraisal. One hundred and sixty-four vaccinations were considered in the present study. In 104 persons the Pirquet test became positive and in 60 persons the reaction remained negative. Among the 104 subjects with positive Pirquet reactions, tuberculosis developed in three, or 3 per cent, while among the 60 with negative reactions, tuberculosis developed in 16, or 26.6 per cent. This criterion has furnished the chief reason for the systematic vaccination with *BCG* of persons with negative Pirquet reactions, since it appeared that these persons are exquisitely susceptible to tuberculous infection. The absence of a positive Pirquet reaction, following vaccination with *BCG*, must be considered as an indication of inadequate vaccination and of the fact that the vaccinated persons' liability to tuberculous infection has remained practically unaltered and revaccination should be done.—*Immunity to Tuberculosis: Relation of Cutaneous Allergy and Resistance to Tuberculous Infection Following Vaccination with BCG, J. Heimbeck, Arch. Int. Med., June, 1932, xlix, 957.*—(*W. I. W.*)

Transfer of Immunological Response of Tuberculous Animals.—Observations previously made suggest that tuberculous lesions themselves play an important rôle in the characteristic immunological response of tuberculous animals. When small doses of an antigen, such as egg-white, were employed, a striking difference was found in the response of animals in which the antigen was injected directly into a tuberculous lesion as contrasted with similarly infected animals in which the antigen was injected elsewhere. The extirpation of the tuberculous lesion as soon as thirty minutes after the antigen had been injected into it did not prevent the characteristic effect upon the development of sensitization and immunity tuberculin-type reaction. The tuberculous lesion itself, therefore, cannot be the site of the characteristic increased antibody production or be directly responsible for the sensitization of the organism.

During the process of absorption of the antigen through the tuberculous focus some modification of it or some combination of it with the elements of the inflammatory lesion must occur. The nature of the change is unknown. The passive transfer of this influence which tuberculous lesions exert upon immunological phenomena is considered in the present article. Is it possible with cells or extracts of a tuberculous process, or with antigen previously exposed to such a lesion, to transfer to other animals the characteristic response of the tuberculous ones? The transfer of tuberculous omenta or tuberculous peritoneal exudate, a few minutes after the injection of egg-white, to the peritoneal cavities of normal pigs was unsuccessful. When a similar transfer was made into the peritoneal cavity of a previously infected animal, in the stage of infection in which the injection of egg-white alone does not produce the characteristic effect, irregular positive results were obtained. A simultaneous injection of egg-white and tubercle bacilli was often successful in these guinea pigs. The experiments suggest that two factors are involved; (1) the effect which the lesion itself produces upon the antigen, and (2) a certain preparation of the organism as a whole occurring during the course of the infection.—*Further Attempts to Transfer the Immunological Response of Tuberculous Animals*, *L. Dienes*, *J. Immunol.*, *July, 1932*, *xxiii*, 29.—(*J. S. W.*)

Nature of Allergy in Tuberculosis.—When foreign proteins are introduced parenterally into the animal body, the effects which they may produce if they reach the tissues are quite familiar in the guise of the various protein "hypersensitive reactions." Depending upon the conditions the reaction may appear in the form of anaphylactic shock, asthma, hay-fever, serum-sickness, "food allergy," the Arthus phenomenon, or the tuberculin and other similar reactions to the protein of bacteria. While these various conditions appear at first sight to be very different from each other, there is persuasive evidence that underlying all of them is the fundamental circumstance that the exposure of the tissues to a bland, undigested foreign protein stimulates the production of a specific antibody, different for each protein, which has the power of reacting with the protein in some way, and that the different forms of hypersensitive reaction are the results of the interaction of antibody and protein antigen under different conditions. The above forms of hypersensitivity may be separated into two groups. In the first group may be placed those cases in which contact with the foreign protein to which the body has been sensitized leads especially to symptoms which result from the spasmodic contraction of smooth-muscle fibres, particularly the fibres of the bronchial or vascular musculature. In the second group, contact with the same protein produces local damage or death of tissue accompanied by acute inflammation. In both groups general constitutional symptoms and even death may occur, if the protein finds its way into the blood-stream in sufficient quantity under proper conditions. It is possible that an identical form of antibody-antigen interaction may be responsible for both of these objectively different manifestations of hypersensitiveness, but for the purpose of clarity, in the present paper, the term "anaphylactic" is restricted to the muscle-spasmodic type of reaction and the term "allergic" to the inflammatory-necrotizing type. Although an animal may be both allergic and anaphylactic to the same protein, it has been shown that allergy may exist without anaphylaxis and *vice versa*. The tissues of a particular tuberculous animal, for example, may be allergic to tuberculoprotein, and yet its smooth muscle may exhibit no tendency to be thrown into contraction (anaphylaxis) when properly exposed to this protein. Another animal may be both allergic and anaphylactic; and a third, by proper treatment, may be anaphylactic to tuberculoprotein without developing any allergy to the protein. Still these facts do not rule out the possibility that both allergy and anaphylaxis may be merely different manifestations of an identical antibody-antigen reaction occurring under different conditions. Furthermore, it seems clear that the anaphylactic reaction must be the result of interaction of antibody with some part of the protein antigen, since the mixture of the serum of an anaphylactic animal with the protein to which the animal is sensitized may produce anaphylactic shock if injected into a normal animal, thus leaving no doubt of the presence of a specific antibody in the circulating blood. Other experiments make it clear that this *circulating* portion of the antibody is not necessary for the spasm of the smooth-muscle characteristic of the anaphylactic reaction. For example, a strip of smooth muscle from an anaphylactic animal, which has been thoroughly perfused to wash it free from plasma, will still exhibit the characteristic contraction *in vitro* on contact with the specific protein. It therefore appears that, in anaphylaxis, an effective amount of antibody is attached directly to the smooth-muscle fibres, and that in addition a residual amount circulates freely in the blood. In the Arthus

phenomenon (the allergic necrotizing-inflammatory, tuberculin type of reaction produced by the injection of a protein, say egg albumen, into the skin of an animal sensitized by previous injections of that protein), it is plain that we are dealing with an antibody-antigen reaction and that at least a part of the specific antibody is free in the circulation, for the allergic sensitivity to egg albumen can be passively transferred to normal animals by the injection of the serum of an animal capable of exhibiting the Arthus phenomenon. It is not yet known, however, whether any antibody is bound to the tissue cells of the animals susceptible to the Arthus' phenomenon, as it is in the case of anaphylaxis, and when we turn to the allergic state that develops in the course of so many bacterial infections our information becomes so meagre that it is only through reasoning from analogy that we can even suggest that an antibody-antigen reaction takes place. All attempts to detect, by the method of passive transfer, a circulating antibody responsible for the allergy arising during bacterial infections have been inconclusive, and no one has attempted to determine whether the isolated tissues of the allergic body, when freed from nervous and circulatory influences, are sensitive to the action of the particular bacterial protein to which the body has become allergic through infection. The present investigation is an attempt to acquire information regarding this matter. It is perfectly clear that, during the process of infection with the tubercle bacillus, the body becomes changed in some way which renders the relatively bland protein of the bacillus capable of acting upon its tissues as a violent necrotizing poison. Whereas it has been shown that the mixture of egg albumen with the serum from an animal sensitized to that protein resulted in the formation of an irritant capable of producing necrosis when injected into normal tissues, no one has been able to demonstrate the formation of a noxious substance when the serum of allergic, tuberculous animals was mixed with tuberculoprotein. What then is the nature of allergy in tuberculosis, since all attempts to demonstrate the existence of a circulating antibody have been unsuccessful or equivocal? It may be that there is in the plasma of the allergic animal an antibody capable of interacting with tuberculoprotein to produce a substance toxic for cells, but that the methods used to demonstrate it have heretofore been inadequate. It may be that the antibody has become attached to the tissue cells and that no appreciable surplus of antibody remains in the circulating blood. It may be that there is an antibody in the plasma of such a nature that its interaction with tuberculoprotein leads to the formation of a substance which is noxious only to the cells of the allergic body which may themselves be altered in some way; in such an event it would obviously be impossible to demonstrate the presence of the antibody by passive transfer to normal animals. Finally, it may be that the occurrence of allergic damage to tissues depends upon nervous or circulatory influence independent of any inherent alterations of the tissue cells at the site of the reaction. Since the *in vitro* attack in which the reactions of isolated strips of smooth muscle are studied has yielded such fundamental information in anaphylaxis, it seemed desirable to try to apply to allergy the method of study of isolated tissues. For this purpose the method of tissue culture seemed particularly suitable, for cells under the conditions of tissue culture are delicate indicators of the presence of cytotoxic substance, they are free from all the complicating nervous and circulatory influences, and the study of free cells is by far the most satisfactory method of deciding such questions as to whether the cells of the allergic body will be damaged by tuberculoprotein in the absence of allergic plasma. In tissue culture, the tuberculoprotein added to the drop of plasma in which the cells are placed cannot escape or suffer uncontrollable dilution and the migrating cells, whether normal or allergic, must therefore wander out into a medium containing in each case the same concentration of the antigen. In tissue culture the washed cells and the plasma of the allergic and the nonallergic animals can be brought together with tuberculoprotein in various combinations. The most important are: (1) washed cells of allergic body + plasma of allergic body + tuberculoprotein; (2) washed cells of allergic body + plasma of normal body + tuberculoprotein; (3) washed cells of normal body + plasma of allergic body + tuberculoprotein; and (4) adequate control of combinations. *Experimental procedures:* Blood was drawn with the aid of heparin from normal guinea pigs and from guinea pigs made allergic by sensitization with avirulent R1 human tubercle bacilli. After centrifugating, the supernatant plasma for each guinea pig was drawn off and the cells obtained and washed with Locke's solution. All manipulations were carried out under strict aseptic precautions. When splenic tissue was used, the animal was opened aseptically after having been bled, and a small piece of spleen removed with sterile instruments. This was cut in Locke's solution into minute frag-

ments and washed. The tuberculins used as the source of the tuberculoprotein in the experiment were diluted with plasma and the most satisfactory concentrations were found to be as follows: Old Tuberculin, 1:60 to 1:80; water extract, 1:5; broth filtrate, 1:20. Dilutions of standard concentrated broth control for O.T. were prepared in the same manner. This broth control is merely glycerinated broth, such as that in which the bacilli are grown in the production of tuberculin, concentrated to the same degree as O.T. To part of the plasma from each animal had now been added a definite and identical amount of tuberculin (in most of the experiments O.T. was used) and to another part the same amount of the concentrated broth control for O.T.; to a part of the plasma nothing was added. It was now possible to place either allergic or washed cells in normal or allergic plasma with or without tuberculin, and tissue cultures were set up utilizing the various combinations mentioned in the table. Contact with the tissue ordinarily caused prompt clotting of the drop of heparinized plasma and migration was very soon under way. The cultures were examined from time to time over a period of several days and records were kept of the degree of migration and the viability of the cells in each separate culture. The damaging effect of the tuberculoprotein on the allergic cells was marked and easy to observe. It was evidenced by a decided inhibition of migration of the allergic cells from the explant into the surrounding tuberculin-containing plasma and by the fact that the relatively few cells that did wander out died in a few hours in contrast to the swarming migration of normal cells into the same medium, most of which remained alive and multiplied for several days or more. On account of some unexplainable variations in the degree of migration and survival, only very marked and highly consistent differences in the behavior of the cells under the different conditions to which they were exposed were accepted as positive evidence of cell damage. The results of the experiments, which comprise nearly 2,000 individual cultures, make it clear beyond any doubt that the washed blood or splenic cells from an allergic animal are hypersusceptible to tuberculin and are readily damaged and killed by amounts of Old Tuberculin, water extract or broth filtrate that do not harm the cells of the normal, nonallergic body. It is furthermore clear that, after allergic hypersensitivity is established, the circulating plasma of the allergic body is not necessary for the toxic action of tuberculin, for the washed allergic cells are killed by it even when suspended in the plasma of a normal animal. This very sensitive method of study of free cells has failed to yield any convincing support for the possibility that, in tuberculin allergy, free antibody, capable of acting on the foreign protein to produce a noxious substance, circulates in the blood in appreciable amount as it does in the case of Arthus phenomenon allergy. In certain cases, in which the combination of allergic plasma and tuberculin seemed to be a somewhat less favorable medium for the normal cells than the combination of normal plasma and tuberculin, the inhibition of migration and death of normal cells in the mixture of allergic plasma and tuberculin was in no instance remotely comparable to the damage suffered by the allergic cells of the same series. It was found that the polymorphonuclear and the monocyte-clasmatocyte cell types were apparently more susceptible to the action of tuberculin than were the cells of the connective tissue. It is possible that the lymphocytes of the allergic body are less susceptible to tuberculin than the monocytes and polymorphonuclear cells. In what the heightened susceptibility of the allergic cells consists cannot be said. It is conceivable that they have undergone some internal alteration that renders them susceptible to the action of tuberculoprotein, but it is much more probable that the change consists in the intimate attachment of antibody to the cells comparable to the fixation of antibody to smooth-muscle cells, which is now generally believed to underlie the condition of anaphylaxis. In contrast to anaphylaxis the authors have not been able, even by the sensitive method of tissue culture, to demonstrate convincingly the existence of appreciable amounts of antibody in the plasma of the tuberculous allergic body, although suggestive evidence of small amounts was occasionally obtained. It is, possible, however, and indeed highly probable that minute amounts of antibody do circulate in the blood-stream of the allergic animal, for antibody formation is apparently not a common property of all cells. In all probability the reticulo-endothelial system is responsible for the formation of antibody; and, if this be true, it is obvious that, since all tissues of the allergic body are hypersensitive, in order for antibody to become attached to distant cells it must travel to them by way of the blood-stream. Just as the surplus of antibody in the blood in anaphylaxis is not necessary for the anaphylactic contraction of smooth muscle, so in tuberculin allergy circulating antibody is not immediately necessary for the allergic damage which occurs in the presence of antigen. If an antibody is involved at all,

and it almost certainly is, it is only the portion bound directly to the tissue cells which is necessary for the occurrence of the local, allergic tissue reaction. *Conclusions:* Washed cells of the allergic, tuberculous body retain their hypersensitivity to tuberculin when isolated from the body in tissue cultures. It is therefore clear that neither circulatory, nervous, nor other mechanisms dependent on the intact body are necessary for the production of allergic damage. Cellular injury and necrosis associated with allergy in tuberculosis result from a change in the individual fixed tissue and blood cells, which renders them hypersensitive to the protein of the tubercle bacillus. This change, in all probability, consists in the intimate attachment of antibody to cells. Free, circulating antibody is not necessary for the production of the immediate local injurious effects of allergy in tuberculosis.—*The Nature of Allergy in Tuberculosis as Revealed by Tissue Culture Studies, A. R. Rich and M. R. Lewis, Bull. Johns Hopkins Hosp., February, 1932, l, 115.*—(*J. S. W.*)

Key to Abstractors

M. P.: Max Pinner, Tucson, Arizona.
R. D.: Richmond Douglass, Loomis, New York.
J. S. W.: J. Stanley Woolley, Loomis, New York.
E. H. K.: E. H. Kerper, Loomis, New York.
W. I. W.: Walter I. Werner, Pontiac, Michigan.
H. J. C.: H. J. Corper, Denver, Colorado.
F. G. P.: Frank G. Petrik, Loomis, New York.
M. E. P.: M. E. Parker, Kalamazoo, Michigan.
H. S. W.: H. S. Willis, Northville, Michigan.